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Effect of including fresh Stylo (*Stylosanthes guianensis, CIAT 184)* and cassava foliage (*Manihot esculenta,* Crantz), fed separately or in a mixture on digestibility, intake, and N retention in growing pigs

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Abstract

Eight individually housed crossbred (Large White x Mong Cai) castrated male pigs of 26 to 30 kg were used to study the effect of fresh stylo (*Stylosanthes guianensis, CIAT 184*) (ST) and cassava foliage (CL), fed alone or in a mixture (MIX), together with a basal diet (Ctrl), on the intake, nutrient digestibility and nitrogen balance. The basal diet and the foliages were fed separately *ad libitum*.

Total feed dry matter (DM) intake was higher when fresh stylo was offered mixed with cassava foliages in the proportion 50:50 (DM basis) (1624 g/day) as compared with fresh stylo (1583 g/day) or cassava foliages (1483 g/day) as the sole supplement and compared to the basal diet (1361 g/day) ($P< 0.05$). There were no significant differences ($P>0.05$) among treatments in digestibility of DM, but N digestibility was lowest for the CL (83.3%) and MIX (83.4%) treatments compared to Ctrl (85.1%).

N retention was higher ($P<0.05$) for the MIX (15 g/day), ST (14 g/day) and CL (13 g/day) treatments than for the Ctrl (11 g/day).

There were no differences in DM digestibility among the foliages, which were in the range 58.9 % (CL) to 65.0 % (ST), nor in the N digestibility, which was in the range 60.5 % (CL) to 73.4 % (FST).

It was concluded that providing *Stylosanthes guianensis* and cassava foliage in a mixture increased DM intake and N retention compared to feeding them as the sole supplement and compared to a basal diet.

Key words: Fresh Stylosanthes guianensis; cassava foliage; Digestibility; Nitrogen balance; Intake; Pigs.

1. Introduction

Cassava (*Manihot esculent,* Crantz) is an important crop in the small farm sector in Laos. There is presently much interest in tropical countries in the use of cassava leaves as a replacement for Soya bean meal and fish meal in pig diets (Preston, 2001). A considerable amount of research has been carried out on using cassava leaf as a protein source for monogastric animals. For example, Devendra (1992) stated that cassava leaf has high crude protein content (170 to 390

g/kg DM). Phuc *et al*. (1996) evaluated the effect of different levels of substitution of soya bean meal by dried cassava leaves (DCL) and found a depression in N digestibility and retention as the level of DCL in the diet increased. In early experiments the leaves were usually fed either dried or ensiled (Phuc *et al*., 1996), to avoid potential toxicity problems due to the cyanogenic glycosides in the fresh cassava leaves (Tewe, 1991). It has been reported that when cassava leaves are harvested together with the roots, it is possible to obtain some 1 to 4 tons DM per hectare (Ravindran, 1995). Therefore from the point of view of availability, this non-conventional tropical source of protein can replace other expensive protein sources in diets for pigs. *Stylosanthes guianensis*, CIAT 184, commonly known as "Stylo 184" (Horne and Stur, 1999) at the present time is widely used in tropical countries (Mannetje and Jones, 1992), and Stylo 184 grown in Thailand produced 12-17 tons DM yield/ha/year with 14-18% crude protein (CP) (Satjipanon *et al*, 1995). Fresh stylo foliage (Stylo CIAT 184) has also been used as a protein supplement for local pigs in Laos, and increased the growth rates when added at up to 6% of the diet DM to a basal diet of maize and rice bran without any negative affects on health (Chanphone and Mikled, 2003). Both cassava and stylosanthes are widely grown in Laos.

The aim of the present experiment was to study the effect of including fresh stylosanthes cassava foliage, fed separately or in a mixture, on digestibility, intake, and N retention in crossbred growing pigs.

2. Materials and Methods

2.1. Location and climate of the study area

The experiment was conducted in the Livestock Research Centre, which is located about 40 km from Vientiane, Lao PDR, at an altitude of 150 m above sea level. The climate in this area is divided into two main seasons: dry and wet. The wet season lasts 6 months from May to October. Annual rainfall averages about 1600 mm and the peak rainfall occurs in the period July to August. The dry season lasts from November to April, and only about 1 to 2% of the annual rainfall occurs during the dry season. The average minimum and maximum temperatures are about 15^{o}C and 32^{o}C, respectively. The experiment was carried out during the months July to August.

2.2. Experimental feeds

The cassava foliages (*Manihot esculenta*, Crantz Rayong Var 72) were harvested from 4 month-old plants grown in plots at the Livestock Research Centre. The leaves were not separated from the petioles and stems, chopped to about 2 to 3 cm lengths and were then spread out in the shade with cross ventilation and wilted and dried for three days before being offered to the pigs. Each morning fresh Stylo 184, at the age of 2 to 3 months, was cut and carried from fields near the pig house, then chopped into 4 to 5 cm lengths, and fed immediately. The dietary treatments included fresh Stylo 184, dried cassava leaves and a mixture of dried cassava leaves and fresh Stylo (50:50) on DM basis, and a basal diet (Table 3). There were thus four treatments:

- Ctrl - Basal diet (*ad-libitum*)
- CL - Basal diet (*ad- libitum*) + cassava foliage (*ab-libitum*)
- ST - Basal diet (*ad-libitum*) + fresh Stylosanthes (*ad-libitum*)
- MIX- Basal diet (*ad-libitum*) + mixture of 50% cassava foliage + 50% Fresh Stylosanthes (*ad-libitum*)

Fresh stylosanthes and cassava foliage and the mixture were offered in separate feeders to the basal diet.

2.3. *Experimental design*

The experiment was done according to a double 4*4 Latin Square arrangement of 4 dietary treatments with eight (Large White x Mong Cai*)* castrate young male pigs with live weights from 26 to 30 kg. The animals were housed in metabolism cages during the whole trial. The metabolism cages (60 x 80 cm) were built to allow the quantitative collection of faeces and urine (photo 1). The characteristics of the cages have been described elsewhere (Chhay Ty *et al.*, 2003). The metabolism cages were installed in an open house. The experimental periods were each of 12 days: Seven days for adaptation to allow the pigs to become familiarized with the new diet and a five-day period for collection of faeces, urine and feed refusals.

The pigs were fed twice daily in equal amounts at 08:00 h and 16:00 h. Feed intake was programmed to be *ad-libitum*, and water was permanently supplied through drinking nipples. The animals were weighed at the beginning of the trial and every twelve days.

Photo 1 Metabolism cages used in the experiment

2.4. Data collection

Urine and faeces of each pig were collected separately and weighed daily every morning and stored at – 20 ^{0}C. Urine was collected in a bucket via a plastic sheet and funnel placed below the cage. The pH was kept below pH 4 by collecting the urine in 10 ml of 10% sulphuric acid in order to prevent nitrogen losses by evaporation of ammonia. The urine and faeces from each animal were collected for five days and at the end of the period the faeces were mixed, ground and representative samples taken for analysis.

2.5. Chemical analysis

DM, CF, NDF, ADF, Ca and determination of feed offered and refused and DM, CF, NDF, ADF, Ca and P in faeces were done by microwave radiation (Undersander *et al*., 1993). Nitrogen in faeces and nitrogen in urine, and in feeds offered and refused, was determined according to the Kjeldahl method (AOAC, 1990). All the analyses were conducted in duplicate.

2.6. Statistical analysis

The data were analyzed by ANOVA using the General Linear Model procedure in MINITAB 13.31 program (2000). Tukey pairwise comparisons were used to determine the differences between treatments with confidence level 95.0%

3. Results and Discussions

3.1. Chemical composition of ingredients and diets

The chemical composition of the ingredients is shown in Table 1. DM contents of maize, rice bran, and broken rice and cassava foliages are similar, as these feed components were dried in the sun before storing. The CP and CF contents of stylo 184 and cassava foliages were higher than maize, rice bran and broken rice. The mean CP content of cassava foliages was 149 g/kg DM (Table 1), which was lower than in the literature, where a typical CP content is around 200 g/kg DM (Eggum 1970; Bui Vanh Chinh *et al.,* 1994). The mean CP content of the Stylo leaves was 191 g/kg DM (Table 1), which is similar to the values of 190 and 220 g/kg DM, reported by Chanphone and Choke (2003) and Bounhong *et al.*, (2002), respectively.

The chemical composition of the experimental diets is shown in Table 3. Crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents of the Ctrl, CL, ST and MIX treatments were 93.5, 99.3, 97 and 98.1 g/kg DM; 219, 225, 230 and 227 g / kg DM and 54, 64, 68, and 66 g/kg DM, respectively. The content of CP in the basal diet (Ctrl) (93.5 g/kg DM) was lower than the range of 145 to 150 g/kg DM, recommended for growing pigs by NIAH (1995) and NRC (1988), although the pigs in our experiment were crosses with an indigenous breed, and would have had a lower requirement for protein compared to the NRC recommendation, which is for improved pigs. The CP content in the basal diet was similar to the typical basal diet for Lao indigenous pigs of 99 g/kg DM (Chanphone and Mikled, 2003).

Table 1 Chemical composition of the dietary ingredients (g/kg on dry basis)

Ingredient	**DM**	**CP**	**CF**	**NDF**	**ADF**	**Ash**	**Ca**	**P**
Maize	900	110	23.6	ND	ND	12.6	6.8	3.8
Rice bran	858	86	119	ND	ND	131	7.8	7.8
Broken rice	848	83	12.5	ND	ND	11.4	3.3	5.8
Stylo 184	230	191	186	404	288	59.4	12.8	2.5
Cassava foliage	808	149	188	315	218	72.0	50.6	40.6

ND: not determined

Table 2 Ingredient and chemical composition of the basal diet

Basal diet*	Proportion as fed, %	DM	CP	CF	g/kg DM
Ingredients					
Maize meal	40	90	44.0	9.4	413
Rice bran	20	85	17.2	23.8	195
Broken rice	39	85	32.3	4.9	382
Vitamin mineral premix	0.5				5
Salt	0.5				5
Total	100		93.5	38.1	1000
N			15.0		
Chemical composition **					
DM					872
CP					93.5
CF					38.1
NDF					219
ADF					54.0
Ash					35.6
Ca					5.5
P					7.5

*Control diet (Ctrl)
**Calculated value (See table 1)

Table 3 Chemical composition of the experimental diets (g/kg DM) **

Item	**Ctrl**	**CL**	**ST**	**MIX**
DM	872	867	833	850
CP	93.5	99.3	97	98.1
CF	38.1	47.1	47	47
NDF	219	225	230	227
ADF	54	64	68	66
Ash	35.6	37.8	37.0	37.4
Ca	5.5	8.4	6.3	7.3
P	7.5	9.5	7.2	8.3

Ctrl: Basal diet; CL: Basal diet *ad libitum* and cassava foliages *ad libitum*; ST: Basal diet ad libitum and fresh stylo *ad libitum*; MIX: Basal diet *ad libitum* and (50:50) cassava foliage, fresh stylo ad libitum
**Calculated value (See Table 2)

3.2. Feed intake

There were significant differences among the experimental diets for DM intake, and CP intake ($P<0.01$), but there were no significant differences among the treatments for DM intake as a percentage of body weight ($P>0.05$) (Table 4) Moreover, there were significant differences among treatments ($P<0.01$) in total DM intake of foliages and total DM intake (Table 4). Daily DM intakes of the cassava foliages, stylosanthes and mixture of the two were 90, 29 and 116 g/day, respectively ($P<0.001$), corresponding to 6.5, 1.9 and 7.7 % of total DM intake, respectively. With addition of the foliages, either alone or a mixture, to the basal diet, the total DM intake, as well as intake of the basal diet itself, increased significantly ($P<0.001$). Total DM

intake was highest in the mixture of stylo and cassava foliages (1624g/day), compared with 1361, 1483, and 1583 g/day for the Ctrl, CL, ST treatments, respectively (P<0.001) (Table 4 and Figure 1). This possibly can be explained by the mixture of cassava foliage and stylo being more palatable than the basal diet or foliage alone. It is also probable that the foliage mixture had a better amino acid balance than the basal diet and the cassava and stylo alone. Another explanation could be that some of the vitamins in the vitamin-mineral premix included in the basal diet could have deteriorated during storage in the hot and humid conditions. Vitamin A in particular loses potency, and green feeds are rich in vitamin A precursors, which may have stimulated intake. However, intake of the stylo was only 1.9% of total DM intake, which was less than the value of 6.4% of total DM intake reported by Chanphone and Mikled (2003) in a similar study carried out in Laos, but with indigenous pigs. In the study of Preston *et al.*, (2004) intake was 156 g/day, compared to only 126 g/day in our experiment. However, DM intake of the cassava leaves in our study was higher than in the experiment of Nguyen Duy Quynh Tram (2003) (57.8 g/day). This was maybe because, the basal diets were different, as in our study the basal diet included maize, rice bran and broken rice, but in the experiment by Nguyen Duy Quynh Tram (2003) the basal diet consisted of broken rice only with would be low palatability than our Basal diet due to content of rice bran and maize meal with a high palatable content than only broken rice, so the feed intake would be increased, then supported our result with higher intake (McDonald *et al.*, 2002).

Table 4 Daily feed dry matter (DM) and crude protein (CP) intakes of pigs fed cassava foliages and fresh stylo, either separately or in a mixture, and a basal diet

	Dietary treatment #					
	Ctrl	**CL**	**ST**	**MIX**	SE	P-value
Intake, g fresh/ day						
Basal diet (Ctrl)	1561^{c}	1598^{b}	1782^{a}	1729^{a}	3.18	0.001
Cassava foliage	0	112	0	113		
Fresh stylosanthes	0	0	125	109		
Total	1561^{c}	1710^{b}	1907^{a}	1951^{a}	7.31	0.001
Intake, g DM/day						
Basal diet	1361^{c}	1393^{b}	1554^{a}	1508^{a}	8.73	0.001
Cassava foliage	0	90	0	91		
Fresh stylosanthes	0	0	29	25		
Total DM, foliage	0	90^{b}	29^{c}	116^{a}		
Total DM	1361^{d}	1483^{c}	1583^{b}	1624^{a}	7.79	0.001
Foliage DM% of total	0	6	2	7		
Body weight of pigs, kg	41.5	43	40.5	41.5		
Intake as a % of BW	3.28	3.45	3.91	3.91	0.08	0.441
Total N, g/day	20^{c}	23^{b}	24^{ab}	25^{a}	0.44	0.001
CP, g/day (N*6.25)	126^{c}	142^{b}	149^{b}	158^{a}	6.02	0.001
CF, g/day	51.3	67.3	72.2	75.7		
NDF, g/day	295	322	353	366		
CP, % of DM intake	9.26	10.2	9.6	10.5		
CF, % of DM intake	3.76	4.53	4.05	4.66		
NDF, % of DM intake	21.67	23.1	22.7	24.3		

[a, b, c] Values within rows with different superscripts letters are significantly different (P <0.05); # see Table 2.

3.3. Digestibility

There were no treatment effects (P = 0.238) on DM digestibility when the foliages where offered together with the basal diet. However, there was a significant difference in N digestibility (Table 5) between the control treatment (85.1%) and the treatment with cassava leaves (83.3%) and the mixture (83.4%) (P<0.05), probably as a result of the high CF, NDF and ADF contents in the foliages compared to the basal diet. The digestibility coefficients for DM and N however were higher than those reported by Bounhong *et al.*, (2002) (84 and 72%, respectively, for DM and N). This is probably, because there was a higher feed intake in the diet in the latter experiment.

Table 5 Digestibility coefficients in pigs fed diets containing cassava foliage and fresh stylo, either separately or in a mixture, and a basal diet.

	Dietary treatment					
	Ctrl	**CL**	**ST**	**MIX**	SE	P-value
DM digestibility	80.6	79.3	80.3	79.5	0.521	0.238
N digestibility	85.1[a]	83.3[b]	84.7[ab]	83.4[b]	0.458	0.033

[a, b, c] Means within rows with different superscripts differ significantly (P<0.05);
sees Table 2.

Table 6 Nitrogen intakes, digested and retained in pigs fed cassava foliages and fresh stylo, either separately or in a mixture, and a basal diet.

	Dietary treatment #					
	Ctrl	**CL**	**ST**	**MIX**	SE	P-value
N balance, g/d						
N intake	20[c]	23[b]	24[ab]	25[a]	0.441	0.000
N in faeces	3.0[c]	3.8[b]	3.7[b]	4.2[a]	0.065	0.000
N in urine	5.9[b]	5.9[b]	6.6[a]	5.7[b]	0.174	0.002
N digested	17[c]	19[b]	20[ab]	21[a]	0.441	0.000
Digestibility, %	84.9	83.5	84.8	83.2	0.459	0.033
N-retention, g/d	11[b]	13[a]	14[a]	15[a]	0.503	0.000
% of N intake	55.3[b]	57.8[b]	57.3[b]	60.4[a]	1.360	0.034
% of N digested	65.2[b]	69.3[b]	67.6[b]	72.6[a]	1.360	0.034

[a, b, c] Mean within rows with different superscripts differ significantly (P <0.05);
see table 2.

Table 7 Digestibility of cassava leaves, stylo and a mixture of the two foliages

	DCL	**FST**	**M**	SE	P-value
DM digestibility, %	58.9	65.7	65.0	19.3	0.964
N digestibility, %	60.5	73.4	63.2	16.8	0.849

DLC: Cassava foliages; FST: Fresh stylo; M: Mixture of DCL and FST
[a, b, c] Means within rows with different superscripts differ significantly (P<0.05)

3.4 Nitrogen retention

There were significant differences among treatments in N intakes, 20 , 23 , 24 and 25 g/day for Ctrl, CL, ST and MIX dietary treatments, respectively ($P<0.01$) (Table 6) due to the higher total DM intakes and the higher N content of the foliages. There was a curvilinear relationship between daily N retention and the proportion of the foliages of the total intake (Figure 2). The values for N retention, and N retention as percentage of N intake and N digested, for the mixture of leaves treatment, were all higher that was reported by Bounhong *et al.*, (2002) and Nguyen Duynh QuynhTram (2003) for similar diets but with "older" cassava and stylo leaves.

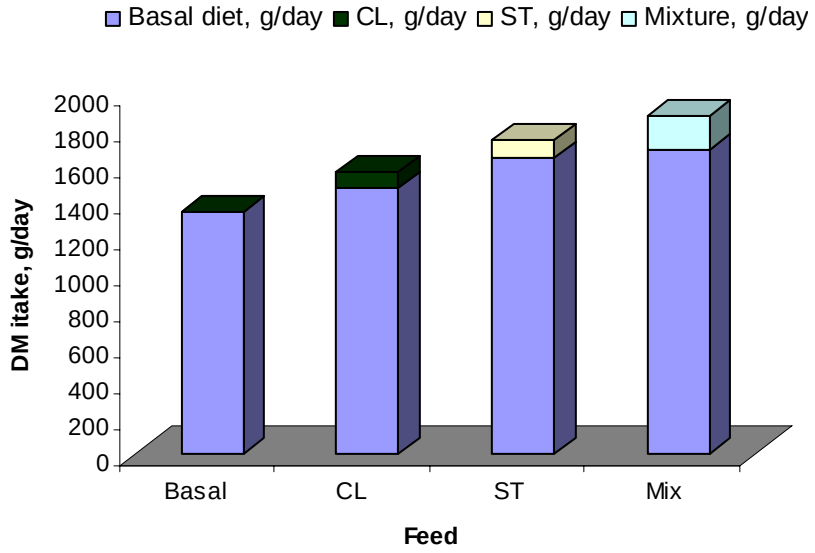

Figure 1 Dry matter intake of cassava foliages (CL), fresh stylosanthes (ST), and a mixture of the two (MIX), and the basal diet (Ctrl)

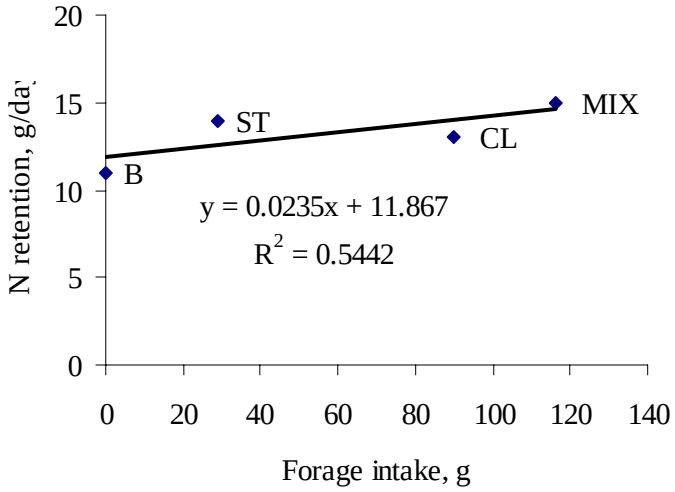

Figure 2 Relationship between N retention and forage intake

4. Conclusions

- Offering dried cassava leaves and fresh stylosanthes *ad libitum* together with a typical low protein basal diet increased intakes of both the basal diet and also overall DM intake, especially when the leaves were offered in a 50:50 mixture.
- N retention was improved relative to the basal diet when the foliages were offered *ad – libitum.*

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